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Reciprocal Nucleopeptides as the Ancestral Darwinian Self-Replicator

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Abstract

Even the simplest organisms are too complex to have spontaneously arisen fully-formed, yet precursors to first life must have emerged *ab initio* from their environment. A watershed event was the appearance of the first entity capable of evolution: the Initial Darwinian Ancestor. Here we suggest that nucleopeptide reciprocal replicators could have carried out this important role and contend that this is the simplest way to explain extant replication systems in a mathematically consistent way. We propose short nucleic-acid templates on which amino-acylated adapters assembled. Spatial localization drives peptide ligation from activated precursors to generate phosphodiester-bond-catalytic peptides. Comprising autocatalytic protein and nucleic acid sequences, this dynamical system links and unifies several previous hypotheses and provides a plausible model for the emergence of DNA and the operational code.

Key words: Initial Darwinian Ancestor; abiogenesis; RNA world; protein world; nucleopeptide replicator; reciprocal replicator; polymerase; ribosome; evolution; early earth; Hypercycle.

Introduction

In contrast to our good understanding of more recent evolution, we still lack a coherent and robust theory that adequately explains the initial appearance of life on Earth (abiogenesis). In order to be complete, an abiogenic theory must describe a path from simple molecules to the Last Universal Common Ancestor (LUCA), requiring only a gradual increase in complexity.

The watershed event in abiogenesis was the emergence of the Initial Darwinian Ancestor

(IDA): the first self-replicator (ignoring dead ends) and ancestral to all life on Earth (Yarus 2011). Following the insights of von Neumann, who proposed the kinematic model of self-replication (Kemeny 1955), necessary features of such a replicator are: Storage of the information for how to build a replicator; A processor to interpret information and select parts; An instance of the replicator.

In order to be viable, any proposal for the IDA's structure must fit with spontaneous emergence from prebiotic geochemistry and

principles of self-replication. Currently, the most dominant abiogenesis theory is the “RNA world”, which posits that the IDA was a self-replicating ribozyme, i.e. an RNA-dependent RNA polymerase (Cech 2012). Although popular, this theory has problems (Kurland 2010). For example, while it is plausible that molecules with the necessary replication characteristics can exist, length requirements seem to make their spontaneous emergence from the primordial milieu unlikely, nor does the RNA world explain the appearance of the operational code (Noller 2012; Robertson and Joyce 2012). Furthermore, it invokes three exchanges of function between RNA and other molecules to explain the coupling of polynucleotide and protein biosynthesis, namely transfer of information storage capability to DNA and polymerase activity to protein as well as gain of peptide synthesis ability. This presents a situation in which no extant molecule continues in the role it initially held. Others have posited peptide and nucleopeptide worlds as solutions.

The peptide world theory proposes a spontaneously occurring self-replicating peptide with RNA synthesis, DNA and the operational code appearing later, and possible self-replicating mechanisms of peptides have been explored (Fox and Harada 1958; Lee et al 1996). Nucleopeptide theories require that the replicator consist of both peptides and nucleic acids and may involve their covalent linkage or (as in our proposal) non-covalent conjugation. Covalently linked

nucleopeptides include nucleobase-containing peptides such as PNA which has been mooted as a possible precursor to the RNA world (Miller 1997) and possible RNA-interacting nucleo- ϵ -peptides have been synthesized (Nelson et al 2000; Roviello et al 2009). Both the peptide world and nucleopeptide theories consist of single molecular classes and therefore suffer the same exchange of function problems as the RNA-world theory. To the best of our knowledge, no single theory has emerged that parsimoniously answers the biggest questions.

Here we build on several foregoing concepts to propose an alternative theory based around a nucleopeptide reciprocal replicator that uses its polynucleotide and peptide components according to their strengths, thus avoiding the need to explain later exchange of function and coupling. We advocate a view of the IDA resulting from a biochemical system which we describe as a dynamical system, i.e. a system of equations describing the changes that occur over time in the self-replicator presented here, and we demonstrate that such an entity is both mathematically consistent and complies with all the logical requirements for life. While necessarily wide in view we hope that this work will provide a useful framework for further investigation of this fundamental question.

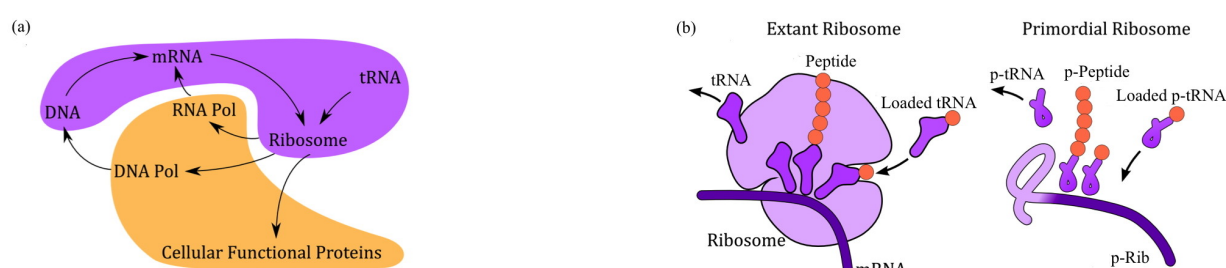


FIG. 1. Replication Schemes. (a) This simplified cellular replication schematic is common to all life today and likely reflects the ancestral form present in LUCA. Shading by molecule type (purple for nucleic acid and orange for protein), reveals a reciprocal nucleopeptide replicator. Although the ribosome is a large nucleoprotein complex, the catalytic centre has been shown to be a ribozyme (Moore and Steitz 2003) and so it is shaded purple in this scheme. (b) Comparison of the method of action of the extant ribosome with the proposed primordial analogue (components are shaded like for like). Today, tRNA molecules (mid purple) loaded with amino acids (orange) bind the mRNA (dark purple) in the ribosome (light purple), which co-ordinates and catalyses the peptidyl-transferase reaction. Although the present day modus operandi is regulated via far more complex interactions than the primordial version, the two schemes are fundamentally similar. Mixed nucleic acid structures, one performing a dual function as primordial mRNA and primordial ribosome (p-Rib) and a second functioning as a primordial tRNA (p-tRNA), provide a system wherein the former structure templates amino acid-loaded molecules of the latter.

Model and Results

Solving the chicken and egg problem

Given that any IDA must have been able to replicate in order to evolve, extant cellular replication machinery is an obvious source of clues to its identity. Common ancestry means that features shared by all life were part of LUCA. By examining the common replication components present in LUCA, and then extrapolating further back to their simplest form, it is possible to reach a pre-LUCA, irreducibly complex, core replicator (Figure 1).

We see that in all cells, the required functions of a replicator are not carried out by a single molecule or even a single class of molecules, rather they are performed variously by nucleic acids (DNA, RNA) and proteins. When viewed by molecular class, the replicator has two components and is reciprocal in nature: polynucleotides rely on proteins for their polymerisation and vice versa. The question of which arose first is a chicken and

egg conundrum that has dogged the field since the replication mechanisms were first elucidated (Giri and Jain 2012). In this work we suggest that, consistent with common ancestry and in contrast with the RNA world theory, the earliest replicator was a two - rather than a one - component system, composed of peptide and nucleic acids.

Assumptions of the model

We postulate that, in a nucleopeptide reciprocal replicator, the use of each component according to its strengths could deliver a viable IDA more compatible with evolution to LUCA replication machinery. Although seemingly more complex than an individual replicating molecule, the resulting unified abiogenesis theory answers many hard questions and is ultimately more parsimonious. The model does not consider in detail the chemistry of how the building blocks that constitute the IDA (short peptides and nucleic acids) came about as these details are covered in the cited literature (see for example (Da

Silva et al 2015; Leman et al 2004; Liu et al 2014; Martin et al 2008; Patel et al 2015; Saladino et al 2012)). Rather we concentrate on the important question of the mathematical validity of the IDA in terms of its ability to sustainably self-replicate, without which it would not be a valid system. In constructing our model, we make the following assumptions:

(i) The existence of random sequences of short strands of mixed nucleic acids (XNA) likely consisting of ribonucleotides, deoxyribonucleotides and possibly other building blocks able to polymerise with nucleotide chains, as well as the existence of random amino acids and short peptides produced abiotically.

For this first assumption we have supposed a pool of interacting amino acids, nucleotides and related small molecules as well as a supply of metal ions, other inorganic catalysts and energy. The precise understanding of the “metabolic” reactions in which these precursor building blocks were formed is in itself an extremely important question but is not considered here as a number of potential early earth conditions and reaction pathways resulting in these outcomes have already been proposed, including the formamide reaction (Saladino et al 2012) and cyanosulfidic chemistries (Patel et al 2015). Recent experimental models of alkaline hydrothermal vents have even succeeded in producing various organic molecules including ribose and deoxyribose (Herschy et al 2014). Pools of pure molecules are unlikely; instead, mixtures would likely have comprised standard and non-standard amino acids as well as XNAs with mixed

backbone architectures, being, in their simplest forms, mixtures of deoxy- and ribonucleotides (Pinheiro et al 2012; Trevino et al 2011) with other building blocks being possible. For simplicity we sometimes refer to XNAs as “polynucleotides”. Such conditions would be conducive to the occasional spontaneous covalent attachment of nucleotides to each other to form longer polymer chains (Da Silva et al 2015).

(ii) The existence of abiotically aminoacylated short XNA strands (primordial tRNAs (p-tRNAs))

The second assumption is potentially troubling as amino acid activation is slow and thermodynamically unfavourable. However, amino acylation has been investigated in some detail and has been shown to be possible abiotically including, in some cases, the abiotic production of activated amino acids (Giel-Pietraszuk and Barciszewski 2006; Illangasekare et al 1995; Lehmann et al 2007; Leman et al 2004; Liu et al 2014; Turk et al 2010). A pool of activated amino acids allows us to presume a fast rate of charging of primordial tRNAs meaning that we can assume that the rate of charged p-tRNA formation is proportional to the concentration of free amino acids. Taken together these data suggest that multiple small amino-acylated tRNA-like primordial XNAs could have arisen. Though likely being XNA in nature, we refer to them as p-tRNA, reflecting

their function. A similar nomenclature applies to p-Rib and p-mRNA.

(iii) *Conditions that allow a codon/anti-codon interaction between two or more charged p-tRNA for sufficient time and appropriate geometry to allow peptide bond formation, i.e. the functionality of a primordial ribosome (p-Rib)*

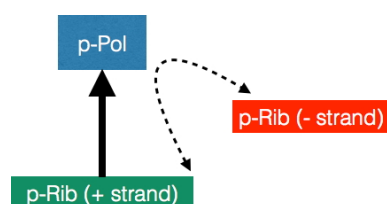


FIG. 2. Models of primitive polymerization reactions. An XNA strand can function like a primordial ribosome (p-Rib) whereby one strand (+ strand) can template the production of a primordial polymerase (p-Pol) as indicated by the solid arrow. The action of this p-Pol is represented by the double-headed dotted arrow whereby it acts on the p-Rib (+ strand) to catalyse synthesis of the complementary sequence (- strand) and also on the - strand to produce more of the + strand.

Our proposed p-Rib is an extreme simplification of the functionality of both the present day ribosome and mRNA (Figure 1). Initially, the p-Rib need only have been a (close to) linear assembly template for the p-tRNAs to facilitate the peptidyl transferase reaction through an increase in local concentration. This mechanism is simple enough to emerge spontaneously and matches exactly the fundamental action of the extant ribosome (Figure 2). The idea that a p-Rib may have an internal template rather than separate mRNA molecules and that an RNA strand could act as a way to bring charged tRNAs together has previously been suggested (Morgens 2013; Schimmel and Henderson 1994; Wolf and Koonin 2007) and is known as an “entropy trap” (Ruiz-Mirazo 2014; Sievers et al 2004). The concept has been demonstrated to be

experimentally viable (Tamura 2003) although in the latter case it is the primordial ribosomal rRNA strand itself that provides one of the two reacting amino acids.

A functional operational system requires preferential charging of particular p-tRNAs to specific amino acids. Although there is evidence for such relationships in the stereochemical theory (Woese 1965; Yarus et al 2009), so far unequivocal proof has been elusive (Koonin and Novozhilov 2009; Yarus et al 2005). However, there is sufficient evidence to suggest at least a separation along grounds of hydrophobicity and charge using just a two-base codon (Biro et al 2003; Knight and Landweber 2000; Rodin et al 2011). Furthermore only a reduced set of amino acids (Angyan et al 2014) - possibly as few as four (Ikehara 2002) - need to have been provided in this way. The “statistical protein” hypothesis proposes that such a weak separation may have been sufficient to produce populations of active peptides (Ikehara 2005; Vetsigian et al 2006). Such “primordial polymerases” (p-Pol) need only have been small (see below) and spontaneous emergence of a template coding loosely for such a sequence seems plausible. The failure rate of such syntheses would be high but a p-Rib using the outlined primordial operational code to produce statistical p-Pol peptides could have been accurate enough to ensure its own survival.

(iv) The viability of a very short peptide sequence to function as an RNA-dependent RNA polymerase

Templated ligation is often proposed as a primordial self-replication mechanism, particularly for primitive replication of nucleic acid in RNA world type scenarios. However, these are associated with a number of problems as mentioned earlier. In addition, extant RNA/DNA synthesis proceeds via terminal elongation (Paul and Joyce 2004; Vidonne and Philip 2009). To be consistent with the mechanism present in LUCA and pre-LUCA, the p-Pol should, preferably, have used a similar process.

During templated ligation, a parent molecule binds and ligates short substrates that must then dissociate to allow further access, but the product has greater binding affinity than the substrates and dissociation is slow. This product inhibition results in parabolic growth and limits the usefulness of templated ligation for replication (Issac and Chmielewski 2002). Conversely, in 1D sliding (or more accurately jumping), the catalyst may dock anywhere along a linear substrate and then diffuse by “hops” randomly in either direction until it reaches the reaction site; a successful ligation reaction has little impact on binding affinity and leaves the catalyst proximal to the next site. For simplicity our model assumes a single binding event between p-Pol and p-Rib followed by multiple polymerization events. A p-Pol proceeding via 1D sliding could catalyze phosphodiester bond formation between

nucleotides bound by Watson and Crick base-pairing to a complementary XNA strand. Because p-Pol activity would be independent of substrate length, a relatively small catalyst could have acted on XNAs of considerable size. From inspection of present day polymerases such a peptide may have included sequences such as DxDGD and/or GDD known to be conserved in their active sites and consisting of the amino acids thought to be amongst the very earliest in life (Iyer et al 2003; Koonin 1991).

In our simple system any such p-Pol must be very short to have any realistic chance of being produced by the primitive components described. We must therefore ask if there is evidence that small (*e.g.* less than 11 amino acid) peptides can have such a catalytic activity. Catalytic activity in general has been demonstrated for molecules as small as dipeptides (Kochavi et al 1997). For polymerase activity in particular, it is known that randomly produced tripeptides can bind tightly and specifically to nucleotides (McCleskey et al 2003; Schneider et al 2000). We suggest that a small peptide could arise with the ability to bind divalent metal ions, p-Rib and incoming nucleotides. It is interesting to note that small peptides can assemble into large and complex structures (Bromley et al 2008; Fletcher et al 2013) with potentially sophisticated functionality: di- and tripeptides can self-assemble into larger nanotubes and intriguingly it has even been suggested that these structures could have acted

as primitive RNA polymerases (Carny and Gazit 2005).

In summary, the essence of the model is that on geological timescales, short linear polynucleotides may have been sufficient to template similar base-pairing interactions to those seen in the modern ribosome with small amino-acylated adapters. Given that the majority of ribosome activity stems from accurate substrate positioning, such templating could be sufficient to catalyze peptide bond formation and to deliver phosphodiester-bond-catalytic peptides. As backbone ligation reactions are unrelated to polynucleotide sequence, these generated primordial enzymes could have acted on a large subset of the available nucleic acid substrates, in turn producing more polynucleotide templates and resulting in an autocatalytic system.

Mathematical Model

The IDA described above is attractive both for its simplicity and continuity with the existing mixed (protein/nucleic acid) replicator system in extant cells. However, the question remains as to whether such a system is mathematically consistent, could avoid collapse and instead become self-sustaining. The number of parameters and variables needed to analyse the system in its full complexity is such that one is led to consider simplified models which nevertheless capture essential features of interest. Here we consider a simple model of RNA-protein self-replication.

(a) Constituents

The main constituents of the simplest model of XNA-protein self-replication considered here (see also Figure 1b and Figure 2) are a pool of free nucleotides and amino acids, polypeptide chains - including a family of polymerases - and polynucleotide chains as well as primordial tRNAs (p-tRNA) loaded with single amino acids.

We introduce some notations. Generically, we consider polymer chains Π made of n types of building blocks labelled $1, \dots, n$. In our models, the polymer chains are polypeptides and polynucleotides, and the building blocks are amino acids and codons respectively. With a slight abuse of language, we call the number of constituents (building blocks) of a polymer chain its *length*. So hereafter, ‘lengths’ are dimensionless. The order in which these constituents appear in any chain is biologically significant, and we encode this information in finite ordered sequences of arbitrary length L denoted $S\{L\} = (s_1, s_2, \dots, s_L)$, whose elements $s_j, j = 1, \dots, L$ label the building blocks forming the chains, in the order indicated in the sequences. Each element s_j in the sequence $S\{L\}$ is an integer in the set $\{1, \dots, n\}$ which refers to the type of building block occupying position j in the chain. There are therefore n^L sequences of length L if the model allows n types of building blocks. For instance, the sequence $S\{5\} = (1, 4, 3, 1, 3)$ in a model with, say, $n = 4$ types of building blocks (amino acids or codons), corresponds to a

polymer chain of length 5 whose first component is a type 1 building block, the second component is a type 4 and so on. Given a sequence $S\{L\}$, we introduce subsequences $S\{L, j\} = (s_1, s_2, \dots, s_j)$ (resp. $\widehat{S\{L, j\}} = (s_{L-j+1}, s_{L-j+2}, \dots, s_L)$), $j = 1, \dots, L$, whose elements are the j leftmost (resp. rightmost) elements of $S\{L\}$. In particular, $S\{L, L\} \equiv \widehat{S\{L, L\}} \equiv S\{L\}$, $S\{L, 1\} = s_1$ and $\widehat{S\{L, 1\}} = s_L$. We write

$$S\{L\} = (S\{L, L-\ell\}, \widehat{S\{L, \ell\}}), \quad 0 < \ell < L.$$

In what follows we sometimes refer to families of polymer chains differing only by their length and obtained by removing some rightmost building blocks from a chain of maximum length L_{\max} . Denoting by Π_ℓ^S a polymer chain of length ℓ and sequence $S\{\ell\}$ or subsequence $S\{L, \ell\}$, both having ℓ elements with $L > \ell$, the family of polymer chains obtained from a chain of maximal length L_{\max} and sequence $S\{L_{\max}\}$ is given by $\{\Pi_\ell^S\}_{\ell=1,2,\dots,L_{\max}}$.

In the specific case of XNA/polynucleotide chains entering our model, we use $\Pi = R$ and the sequences are generically labelled as $\alpha\{\ell\}$. Their elements correspond to types of codons, and the complementary codon sequences in the sense of nucleic acids complementarity are $\bar{\alpha}\{\ell\}$. Therefore a large class of XNA strands of length ℓ and sequence $\alpha\{\ell\}$ are denoted by R_ℓ^α , and in particular, $R_1^{\alpha_1}$ is a codon of type α_1 . Besides the generic sequences $\alpha\{\ell\}$ introduced above, a sequence denoted $\pi\{L_{\max}\}$,

together with its subsequences $\pi\{L_{\max}, \ell\}$ and $\pi\{\widehat{L_{\max}}, \ell\}$ for $\ell = 1, \dots, L_{\max}$ play a specific role: they correspond to polynucleotide chains that template the polymerisation of a family of primordial peptide polymerases (p-Pol) through a process described in the next subsection, see also Figure 3. Using $\Pi = P$ to denote polypeptide chains, this family of polymerases derived from $P_{L_{\max}}$ of maximal length L_{\max} , is $\{P_\ell^\pi\}_{\ell=2,\dots,L_{\max}}$. These polymerases are such that $P_\ell^\pi = P_{\ell-1}^\pi + P_1^{\pi_\ell}$, with $P_1^{\pi_\ell}$ an amino acid π_ℓ . We use the notation P^π for a generic polymerase in the family. Alongside these polymerases, generic polypeptide chains of length ℓ and sequence $\alpha\{\ell\}$ are labelled as P_ℓ^α . Proteins of length 1, $P_1^{\alpha_1}$, are single amino acids of type α_1 .

(b) RNA-Protein replication scenario

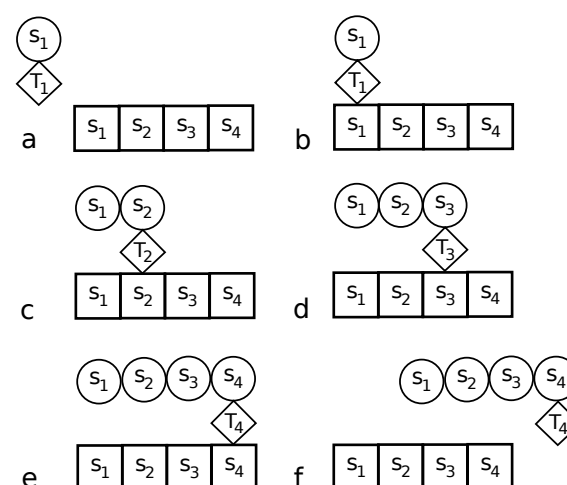


FIG. 3. Mechanism (B): Polypeptide polymerisation in our model. The square boxes represent the codons of a polynucleotide chain (here, of length $L=4$) and the circles represent amino acids. The p-tRNA molecules are labelled T_1, \dots, T_4 .

The scenario relies on three types of mechanisms:

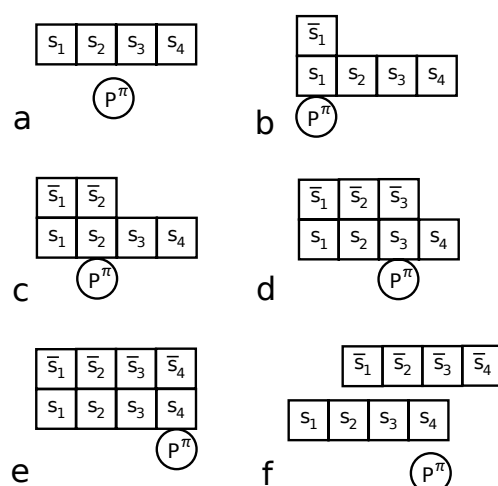


FIG. 4. First phase of Mechanism (C): Polymerisation of the complementary polynucleotide chain $R_L^{\bar{S}}$ catalysed by a primordial polymerase P^π .

(A) The *spontaneous* polymerisation of polynucleotide and polypeptide chains, assumed to occur at a very slow rate, and their depolymerisation through being cleaved in two anywhere along the chains at a rate independent of where the cut occurs.

(B) The *non-spontaneous* polypeptide polymerisation occurring through a polynucleotide chain R_L^S on which several p-tRNA molecules loaded with an amino acid dock and progressively build the polypeptide chain. More precisely, each codon of type s of the polynucleotide chain binds with a p-tRNA, itself linked to an amino acid of type s . Note that we assume the same number n of types of codons and amino acids. This leads to a chain of amino acids matching the codon sequence $S\{L\}$ of the polynucleotide chain. The process is illustrated in Figure 3 for a polypeptide

chain of length $L=4$ and amino acid sequence $S\{4\}=(s_1,s_2,s_3,s_4)$.

(C) The duplication of a polynucleotide chain R_L^S , of length $L \geq \ell_{\pi\min}$, as a two-step process. In the first step, a polypeptide polymerase P^π , obtained by polymerisation via mechanism (B) using a polynucleotide R_L^π , scans the polynucleotide chain R_L^S to generate its complementary polynucleotide chain $R_L^{\bar{S}}$. This is shown in Figure 4. The resulting polynucleotide chain $R_L^{\bar{S}}$ is then used to generate a copy of the original polynucleotide chain R_L^S via the same mechanism (C).

The replicator crudely operates as follows:

- Mechanism (A) provides a small pool of polymer chains; among them, one finds short strands of XNA with dual function (p-mRNA and p-Rib)
- Mechanism (B) provides polypeptide chains, including the polymerases (p-Pol, called P^π here), by using the XNA produced through Mechanism (A) and Mechanism (C)
- P^π are involved, through Mechanism (C), in the duplication of polynucleotides present in the environment, including the strands of XNA that participate in the very production of P^π

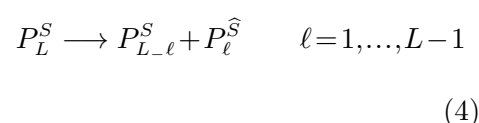
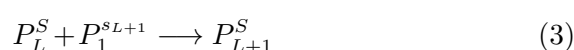
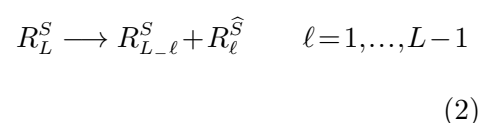
(c) *Reactions driving the replication and physical parameters*

For simplicity, we consider the polymerisation of polypeptide chains and the duplication of polynucleotide chains as single reactions where the

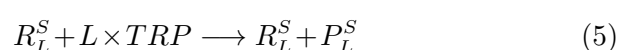
reaction rates take into account all sub-processes as well as failure rates.

This leads to the following schematic reactions:

Mechanism (A)



Mechanism (B)



Mechanism (C)



where TRP denotes primordial tRNA loaded with a single amino acid.

The parameters for these reactions are (see the Supplementary Information for more details on the estimation of the parameter values):

- K_R^+ : polymerisation rate of polynucleotide chains [Equation (1)]; we have estimated the catalysed XNA polymerisation rate to be $4.2 \times 10^{-7} \text{mol}^{-1} \text{m}^3 \text{s}^{-1}$.
- K_R^- : depolymerisation rate of polynucleotide chains (hydrolysis) [Equation (2)]; taken to be $8 \times 10^{-9} \text{s}^{-1}$.
- K_P^+ : polymerisation rate of polypeptide chains [Equation (3)]; we have estimated it to be $2.8 \times 10^{-21} \text{mol}^{-1} \text{m}^3 \text{s}^{-1}$.

- $K_{P,S,L}^-$: depolymerisation rate of polypeptide chains of length L and sequence S [Equation (4)]; we have estimated it to be in the range $4 \times 10^{-11} \text{s}^{-1} - 5.1 \times 10^{-6} \text{s}^{-1}$.
- $k_{P,L}^+$: polymerisation rate of a polypeptide of length L from the corresponding polynucleotide chain [Equation (5)]. It is reasonable to assume that $k_{P,L}^+ = k_{P,1}^+ / L$ and we have estimated $k_{P,1}^+$ to be $0.1 \text{mol}^{-1} \text{m}^3 \text{s}^{-1}$.
- Z : the rate at which a polymerase attaches to a polynucleotide chain [Equation (6)] which we have estimated to be $10^6 \text{mol}^{-1} \text{m}^3 \text{s}^{-1}$.
- h_R : the rate of attachment of a free polynucleotide to a polynucleotide chain attached to a p-Pol [Equation (6)]. We have estimated it to be $10^6 \text{mol}^{-1} \text{m}^3 \text{s}^{-1}$.
- k_{step} : the rate at which a polymerase moves by one step on the polynucleotide [Equation (6)]. We have estimated it to be in the range $2 \times 10^{-2} \text{s}^{-1}$ to $4 \times 10^{-5} \text{s}^{-1}$.

We now argue that the three parameters Z, h_R and k_{step} enter the dynamical system for the polymer concentrations in our model as two *physical* combinations denoted $\mathcal{K}(L)$ and \mathcal{P}_b that we describe below.

First recall that we assume the existence of a pool of nucleotides, amino acids and p-tRNA. The amount of *free* nucleotides and amino acids is taken to be the difference between the total amount of these molecules and the total amount of

the corresponding polymerised material, ensuring total conservation.

We denote the concentration of polypeptide and polynucleotide chains respectively by $P_L^\alpha, P_L^\pi, P_L^{\bar{\pi}}$ and $R_L^\alpha, R_L^\pi, R_L^{\bar{\pi}}$, all expressed in molm^{-3} . In particular, P_1 and R_1 are the concentrations of each type of free amino acids and nucleotides respectively, and we assume, for simplicity, that all types of amino acids/codons are equally available.

We also assume that the amount of loaded p-tRNA, $C_{\text{p-tRNA}}$, remains proportional to the amount of free amino acids and that the concentration of p-tRNA is larger than P_1 so that most amino acids are loaded on a p-tRNA. With these conventions, one has

$$C_{\text{p-tRNA}} = k_t P_1 \quad \text{with} \quad k_t \approx 1. \quad (7)$$

Total reaction rate $\mathcal{K}(L)$ of polynucleotide polymerisation

If a complex reaction is the result of one event at rate K , and m other, identical, events at rate k , the average time to complete the reaction is the sum of the average times for each event. Hence the reaction rate is given by

$$\tilde{\mathcal{K}}(K, k, m) = \left(\frac{1}{K} + \frac{m}{k} \right)^{-1} = \frac{Kk}{mK + k}. \quad (8)$$

One such complex reaction in our model is the polymerisation of a polynucleotide chain of length L , say, from its complementary chain (second phase of Mechanism (C)). Polymerases are characterised by the polymerising efficiency which, we assume, increases with ℓ , up to L_{max} . The first step in polymerisation requires

a polymerase to attach itself to the template polynucleotide. This is only possible if the template polynucleotide has a minimum length, which we assume to be $\ell_{\pi\text{min}}$. In the following, we assume that polymerases can polymerise polynucleotide chains of any length greater or equal to $\ell_{\pi\text{min}}$. The corresponding reaction rate is given by ZP_ℓ^π for a polymerase of length $\ell \geq \ell_{\pi\text{min}}$.

The free nucleotides must then attach themselves to the polynucleotide-polymerase complex and the polymerase must move one step along the polynucleotide. The rate for each of these L steps is

$$k_{R+} = \frac{k_{\text{step}} h_R R_1}{k_{\text{step}} + h_R R_1}, \quad (9)$$

and hence, the rate of polymerisation for a polynucleotide of length L and polymerase of length ℓ is $\tilde{\mathcal{K}}(ZP_\ell^\pi, k_{R+}, L)$. However, it is assumed that polymerases of several lengths are available and therefore, the total rate is given by

$$\mathcal{K}(L) = \begin{cases} \sum_{\ell=\ell_{\pi\text{min}}}^{L_{\text{max}}} \tilde{\mathcal{K}}(ZP_\ell^\pi, k_{R+}, L) W_\ell, & L \geq \ell_{\pi\text{min}} \\ 0 & L < \ell_{\pi\text{min}}, \end{cases} \quad (10)$$

where it is understood that $\ell_{\pi\text{min}}$ is the lower bound length for polymerase activity and W_ℓ is a quality factor given by

$$W_\ell = \begin{cases} \frac{\ell - \ell_{\pi\text{min}} + 1}{\ell_{\pi\text{max}} - \ell_{\pi\text{min}} + 1} & \ell_{\pi\text{min}} \leq \ell \leq \ell_{\pi\text{max}} \\ 1 & \ell_{\pi\text{max}} < \ell \leq L_{\text{max}}. \end{cases} \quad (11)$$

Indeed, we expect long polymerases to be more efficient, so W_ℓ is taken to increase with ℓ in the range $\ell_{\pi\text{min}} \leq \ell \leq \ell_{\pi\text{max}}$, while polymerases of length $\ell > \ell_{\pi\text{max}}$ have the same level of activity as those with length $\ell = \ell_{\pi\text{max}}$ i.e. $W_{\ell > \ell_{\text{max}}} = 1$.

To avoid proliferation of parameters in our simulations, we have taken $\ell_{\pi\max}=L_{\max}$, where L_{\max} is the maximal polynucleotide chain's length.

Binding probability \mathcal{P}_b of a polynucleotide and a polymerase of length L

First note that it takes L times longer to synthesise a polypeptide chain of length L from its corresponding polynucleotide chain than it takes for one amino acid to bind itself to the polynucleotide. The rate is thus given by $k_{P,L}^+ P_1 = (k_{P,1}^+/L) P_1$.

We now offer some considerations on depolymerisation. We assume that if a polymer Π_L^S depolymerises, it does so by (potentially consecutive) cleavings. In the first step, Π_L^S can cleave in $L-1$ different positions, resulting in two smaller chains L_1, L_2 with $L=L_1+L_2$ and $1 \leq L_{1,2} \leq L-1$. This is the origin of the factor $(L-1)$ in the terms describing the depolymerisation of polymer chains in the dynamical systems equations presented in the next subsection.

The concentration variations resulting from such depolymerisations must be carefully evaluated. A polymer Π_L^S of length L and sequence S , where S stands for any of α , π or $\bar{\pi}$, can be obtained by cleaving a polymer $\Pi_\ell^{\tilde{S}}$ of length $\ell > L$ and sequence $\tilde{S}=(S, T)$ where T is a sequence of length $\ell-L$. Similarly it can be obtained by cleaving $\Pi_\ell^{\tilde{S}'}$ of sequence $\tilde{S}'=(T', S)$ where T' is also of length $\ell-L$. If the rate of

cleaving, K_Π^- , is assumed to be independent of the polymer length, and since there are $n^{\ell-L}$ different sequences T and T' , where n is the number of amino acid or codon *types*, the rate of concentration variation of polymers of length L resulting from the depolymerisation of longer polymers is

$$\sum_{\ell=L+1}^{L_{\max}} n^{\ell-L} K_\Pi^- \Pi_\ell^{\tilde{S}} + \sum_{\ell=L+1}^{L_{\max}} n^{\ell-L} K_\Pi^- \Pi_\ell^{\tilde{S}'}. \quad (12)$$

Recall that we use the same notation for the concentration of a polymer of sequence S and length L and the polymer itself, namely Π_L^S , and Π is supposed to be set to $\Pi=P$ or $\Pi=R$ in our model. As already stressed, we assume polymers have at most length L_{\max} . Finally, when the concentrations $\Pi_L^{\tilde{S}}$ and $\Pi_L^{\tilde{S}'}$ are equal, [Equation (12)] can be rewritten as

$$2 \sum_{\ell=L+1}^{L_{\max}} n^{\ell-L} K_\Pi^- \Pi_\ell^{\tilde{S}}. \quad (13)$$

The depolymerisation of polymerase P_L^π requires special treatment. When P_L^π depolymerises, it generates a polymerase P_ℓ^π with $\ell < L$. On the other hand, any P_L^π can be obtained through depolymerisation of one of $2n$ types of polymers of length $L+1$, one of which being P_{L+1}^π and the remaining $2n-1$ being of type P_{L+1}^α with $\alpha\{L+1\}=(\pi\{L\}, \alpha_{L+1}), \alpha_{L+1} \neq \pi_{L+1}$, or $\alpha\{L+1\}=(\alpha_1, \pi\{L\})$ with α_1 any of the n types of amino acids. More generally, they can be obtained from $P_{L+\ell'}^\pi$ and $2n^{\ell'}-1$ polymers of type $P_{L+\ell'}^\alpha$ where $\ell' \geq 1$ and $\alpha\{L+\ell'\}=(\pi\{L\}, \alpha_{L+1}, \dots, \alpha_{L+\ell'})$ with $\alpha_j \neq \pi_j, j=L+1, \dots, L+\ell'$, or $\alpha\{L+\ell'\}=(\alpha_1, \dots, \alpha_{\ell'}, \pi\{L\})$ for any type $\alpha_j, j=1, \dots, \ell'$. The

same is true for the corresponding polynucleotide chains.

When the polymerase is bound to a polynucleotide, it becomes more stable either through induced folding of a (partially) unfolded sequence, or through the inaccessibility of bound portions, or both. We thus define $F_\pi(\ell)$ as the depolymerisation reduction coefficient for the bound polymerase of length ℓ , with that reduction coefficient being 1 when no depolymerisation occurs at all. We estimate it to be

$$F_\pi(\ell) = \begin{cases} 1 - e^{-\frac{\ell - \ell_{\pi\min} + 1}{\lambda}} & \ell \geq \ell_{\pi\min} \\ 0 & \ell < \ell_{\pi\min} \end{cases} \quad (14)$$

with $\lambda > 0$ a parameter controlling how much of the polymerase is stabilised. The term $(\ell - \ell_{\pi\min} + 1)/\lambda$ can be interpreted as a Boltzmann factor with a free energy expressed in units of $k_B T$. The hydrogen bond binding energy between RNA and a polypeptide is approximately 16 kJ/mol [(Dixit et al, 1999)], so assuming that the number of such hydrogen bonds between the polymerase and the polynucleotide is $\ell - \ell_{\pi\min} + 1$, one has $\lambda \approx 0.15$.

The binding rate of a polymerase to a polynucleotide R_M^α of length M and sequence α is $k_{b,M} = Z R_M^\alpha n^M$ where n^M is the total number of polynucleotides of length M . The probability that a polymerase of length L binds to a polynucleotide of length M is therefore given by

$$\tilde{\mathcal{P}}_{b,M} = \frac{k_{b,M}}{\sum_{m=2}^{L_{\max}} k_{b,m}}. \quad (15)$$

The total time the polymerase remains bound to a polynucleotide of length M is estimated to be M/k_{R+} . Therefore the probability \mathcal{P}_b for a polymerase to be bound is given by the average binding time divided by the sum of the average binding time and the average time needed to bind:

$$\mathcal{P}_b = \frac{\sum_{M=2}^{L_{\max}} \frac{M}{k_{R+}} \tilde{\mathcal{P}}_{b,M}}{\sum_{M=2}^{L_{\max}} \left(\frac{M}{k_{R+}} \tilde{\mathcal{P}}_{b,M} \right) + \frac{1}{\sum_{m=2}^{L_{\max}} k_{b,m}}}. \quad (16)$$

As a result the polymerase depolymerisation rate will be

$$\begin{aligned} K_{P,\alpha,L}^- &= K_P^-, \\ K_{P,\bar{\pi},L}^- &= K_P^-, \\ K_{P,\pi,L}^- &= K_P^-(1 - \mathcal{P}_b F_\pi(L)). \end{aligned} \quad (17)$$

(d) Equations

For any chain of length ℓ , our model considers the concentrations of polynucleotides and polypeptides corresponding to the polymerase sequence π , its complementary sequence $\bar{\pi}$ and the generic sequences α . We assume that the concentrations of polynucleotides and polypeptides of a specific length, bar the polymerase and its complementary sequence, are identical. For the chains that share the first ℓ elements of their sequence with those of the polymerase (or its complementary chain), and differ in all other elements, this is only an approximation, but it is nevertheless justified, as the concentrations of these polymers only differ slightly from those of polymers with sequences of type α , and their contribution to the variation of

the polymerase concentration is expected to be small.

The variations in polymer concentrations as time evolves are governed in our model by a system of ordinary differential equations. In the equations, L is the length of the polymer chains, spanning all values in the range $1 < L \leq L_{\max}$ where L_{\max} is the maximal length of polypeptide and polynucleotide chains. We thus have a system of $6 \times (L_{\max} - 1)$ equations. We recall that n is the number of codon types, assumed to be equal to the number of amino acid types.

$$\begin{aligned}
\frac{dR_L^\pi}{dt} &= K_R^+ R_1 R_{L-1}^\pi - n K_R^+ R_1 R_L^\pi \\
&\quad + \sum_{\ell=L+1}^{L_{\max}} [K_R^- R_\ell^\pi + (2n^{\ell-L} - 1) K_R^- R_\ell^\alpha] \\
&\quad - (L-1) K_R^- R_L^\pi + \mathcal{K}(L) R_L^\pi \\
\frac{dR_L^{\bar{\pi}}}{dt} &= K_R^+ R_1 R_{L-1}^{\bar{\pi}} - n K_R^+ R_1 R_L^{\bar{\pi}} \\
&\quad + \sum_{\ell=L+1}^{L_{\max}} [K_R^- R_\ell^{\bar{\pi}} + (2n^{\ell-L} - 1) K_R^- R_\ell^\alpha] \\
&\quad - (L-1) K_R^- R_L^{\bar{\pi}} + \mathcal{K}(L) R_L^{\bar{\pi}} \\
\frac{dR_L^\alpha}{dt} &= K_R^+ R_1 R_{L-1}^\alpha - n K_R^+ R_1 R_L^\alpha \\
&\quad + 2 \sum_{\ell=L+1}^{L_{\max}} n^{\ell-L} K_R^- R_\ell^\alpha - (L-1) K_R^- R_L^\alpha \\
&\quad + \mathcal{K}(L) R_L^\alpha \\
\frac{dP_L^\pi}{dt} &= K_P^+ P_1 P_{L-1}^\pi - n K_P^+ P_1 P_L^\pi \\
&\quad + \sum_{\ell=L+1}^{L_{\max}} [K_P^- (1 - \mathcal{P}_b F_\pi(L)) P_\ell^\pi \\
&\quad \quad + (2n^{\ell-L} - 1) K_P^- P_\ell^\alpha] \\
&\quad - (L-1) K_P^- (1 - \mathcal{P}_b F_\pi(L)) P_L^\pi + k_{P,L}^+ P_1 R_L^\pi
\end{aligned}$$

$$\begin{aligned}
\frac{dP_L^{\bar{\pi}}}{dt} &= K_P^+ P_1 P_{L-1}^{\bar{\pi}} - n K_P^+ P_1 P_L^{\bar{\pi}} \\
&\quad + \sum_{\ell=L+1}^{L_{\max}} [K_P^- P_\ell^{\bar{\pi}} + (2n^{\ell-L} - 1) K_P^- P_\ell^\alpha] \\
&\quad - (L-1) K_P^- P_L^{\bar{\pi}} + k_{P,L}^+ P_1 R_L^{\bar{\pi}} \\
\frac{dP_L^\alpha}{dt} &= K_P^+ P_1 P_{L-1}^\alpha - n K_P^+ P_1 P_L^\alpha \\
&\quad + 2 \sum_{\ell=L+1}^{L_{\max}} n^{\ell-L} K_P^- P_\ell^\alpha - (L-1) K_P^- P_L^\alpha \\
&\quad + k_{P,L}^+ P_1 R_L^\alpha.
\end{aligned} \tag{18}$$

Alongside the seven physical parameters $\{K_R^\pm, K_P^\pm, h_{P,L}^+, \mathcal{K}(L), \mathcal{P}_b\}$ appearing in the differential equations above, we need to consider two parameters yielding the ‘initial’ concentrations of amino acid and nucleotide inside the system, namely $\rho_p \equiv P_1(t=0)$ and $\rho_r \equiv R_1(t=0)$. In the absence of actual data for these quantities, we explore a range of realistic values in the analysis of our model. The concentration of free amino acids and nucleotides at any one time is then given by $P_1(t) = \rho_p - \sum_{L=2}^{L_{\max}} [(n^L - 2) P_L^\alpha(t) + P_L^\pi(t) + P_L^{\bar{\pi}}(t)]$ and $R_1(t) = \rho_r - \sum_{L=2}^{L_{\max}} [(n^L - 2) R_L^\alpha(t) + R_L^\pi(t) + R_L^{\bar{\pi}}(t)]$ respectively, with $P_L^S(0) = R_L^S(0) = 0$ for any value of L in the range $2 \leq L \leq L_{\max}$ and sequence $S = \alpha, \pi, \bar{\pi}$.

Results

The system of equations [Equation (18)] is non-linear and too complex to solve analytically. We therefore analyse it numerically, starting from a system made entirely of free nucleotides, amino acids, as well as charged p-tRNA, and letting

the system evolve until it settles into a steady configuration.

The main quantities of interest are the relative concentrations of the polymerase (ρ_π) and of the α peptide chains (ρ_α). We have

$$\rho_\pi = \sum_{\ell=\ell_{\pi\min}}^{L_{\max}} P_\ell^\pi \quad \text{and} \quad \rho_\alpha = \sum_{\ell=\ell_{\pi\min}}^{L_{\max}} P_\ell^\alpha, \quad (19)$$

and evaluate the ratios

$$Q_1 = \frac{\rho_\pi}{\rho_\alpha} \quad \text{and} \quad Q_{2,\ell} = \frac{P_\ell^\pi}{P_\ell^\alpha} \quad (20)$$

while monitoring the evolution of each quantity over time. Q_1 corresponds to the relative amount of polymerase of any length compared to other proteins (for a specific arbitrary sequence α), while $Q_{2,\ell}$ corresponds to the relative amount of polymerase of length ℓ compared to an arbitrary protein of length ℓ . Unit ratios indicate that the polymerase has not been selected at all, while large values of Q_1 or $Q_{2,\ell}$ on the other hand indicate a good selection of the polymerase.

The complexity of the system [Equation (18)] also lies in the number of free parameters it involves. A systematic analysis of the high-dimensional parameter space is beyond the scope of this article, and we therefore concentrate on the analysis and description of results for a selection of parameter values that highlight potentially interesting behaviours of our model.

Recall that our model assumes that the number n of different amino acids is equal to the number of codon types, and throughout our numerical work we have set $n=4$. Note that the word ‘codon’ here is used by extension. Indeed, there

are four different nucleic acids in our model and the ‘biological’ codons are made of two nucleic acids, bringing their number to sixteen. However, they split into four groups of four, each of which encoding one of the four amino acids. From a mathematical modelling point of view, this is completely equivalent. It is well accepted that early proteins were produced using a reduced set of amino acids (Angyan et al 2014). The exact identity and number is unclear though experimental work has shown that protein domains can be made using predominantly five amino acids (Riddle et al 1997) while the helices of a 4-alpha helix bundle were made using only 4 amino acids (Regan and DeGrado 1988). We have used mostly $\ell_{\pi\min}=7$ and $\ell_{\pi\max}=L_{\max}=10$, but have investigated other values as well (see the Supplementary Information section).

While these figures are somewhat arbitrary, an $\ell_{\pi\min}$ of 7 was chosen on the assumption that the functional p-Pol would have some forms of stable structural motif and this number corresponds to the typical minimum number of amino acids required to produce a stable, folded alpha helix structure (Manning et al 1988). The choice of $L_{\max}=10$ is based on the fact that while the polymer peptide chains could be significantly longer, they would need correspondingly long polynucleotide sequences to encode them, which becomes increasingly unlikely as lengths increase. Furthermore, we expected polymers of length 10 to have very low

concentrations, a hypothesis confirmed by our simulations. We have nevertheless investigated larger values of L_{\max} as well, and found little difference, as outlined below.

In a first step, guided by data on parameter values gleaned from the literature and gathered in the Supplementary Information section, we set

$$\begin{aligned} K_R^+ &= 4.2 \times 10^{-7} \text{mol}^{-1} \text{m}^3 \text{s}^{-1}, \\ K_R^- &= 8 \times 10^{-9} \text{s}^{-1}, \\ K_P^+ &= 2.8 \times 10^{-21} \text{mol}^{-1} \text{m}^3 \text{s}^{-1}, \\ K_P^- &= 4 \times 10^{-11} \text{s}^{-1} \\ k_{P,1}^+ &= 0.1 \text{mol}^{-1} \text{m}^3 \text{s}^{-1}, \\ h_R &= 10^6 \text{mol}^{-1} \text{m}^3 \text{s}^{-1}, \\ Z &= 10^6 \text{mol}^{-1} \text{m}^3 \text{s}^{-1}, \\ \lambda &= 0.15, \\ k_{\text{step}} &= 4 \times 10^{-5} \text{s}^{-1}. \end{aligned} \quad (21)$$

We let the system evolve under a variety of initial concentrations of free amino acids and nucleotides, ρ_p and ρ_r , in the range $10^{-5} - 0.1 \text{mol m}^{-3}$, and with all polymer concentrations set to 0. We monitored the concentration of all polymers, in particular the concentration of polymerase ρ_π and its ratio to the concentration of α polypeptide chains, Q_1 . In most cases we found that the nucleotides polymerised spontaneously (Mechanism (A)) in small amount and this led, indirectly, to the polymerisation of the polypeptides, including the polymerases (Mechanism (C)). The polymerases then induced further polymerisation of the polynucleotides (Mechanism (B)) and the system slowly equilibrated.

The end result was an excess of polymerase of all lengths compared to α polypeptide chains with $Q_1 = 786$ for all initial concentrations $\rho_p = \rho_r \geq 0.001 \text{mol m}^{-3}$ (Figure 5). Moreover the total amount of polymerase reached, for initial concentration of free amino acids ρ_p , was a concentration of approximately $4 \times 10^{-4} \times \rho_p$ (as illustrated by the bottom 2 rows in Table I). The concentration of polymerase of length 10, on the other hand, was very small $P_{10}^\pi = 6.3 \times 10^{-14} \text{mol m}^{-3}$ for but $Q_{2,10} = 5.9 \times 10^{18}$ was very large, effectively showing that the only polypeptide chain of length $L_{\max} = 10$ was the polymerase.

We found hardly any polymerisation of the polymerase when $\rho_p = \rho_r = 0.0009 \text{mol m}^{-3}$, with $\rho_\pi \approx 1.4 \times 10^{-14} \text{mol m}^{-3}$ and $Q_1 = 12.4$, while with $\rho_p = \rho_r = 0.001 \text{mol m}^{-3}$, we obtained $\rho_\pi \approx 3.9 \times 10^{-7} \text{mol m}^{-3}$ and $Q_1 = 786$ (fig 5 a). This highlights a very sharp transition at a *critical concentration* $\rho_{p,c}$ above which polymerases are generated. We summarise the data in Table 1.

We then fixed the initial concentration ρ_p to four different values and varied ρ_r to identify the critical initial concentration of nucleotides necessary for the production of polymerases. The results in Table 2 show that the critical concentration $\rho_{r,c}$ is nearly constant and of the order of $10^{-3} \text{mol m}^{-3}$ for a very wide range of amino acid initial concentrations.

Many of the parameters we have used were estimated or measured in conditions which, in

Table 1. Effect of initial concentrations on polymerase production

$\rho_p(\text{mol m}^{-3})$	$\rho_r(\text{mol m}^{-3})$	$\rho_\pi(\text{mol m}^{-3})$	Q_1	polymerase production
2×10^{-4}	2×10^{-4}	2.810^{-19}	1.0008	insignificant
9×10^{-4}	9×10^{-4}	1.410^{-14}	12.4	insignificant
10^{-3}	10^{-3}	3.9×10^{-7}	786	yes
10^{-1}	10^{-1}	3.9×10^{-5}	786	yes

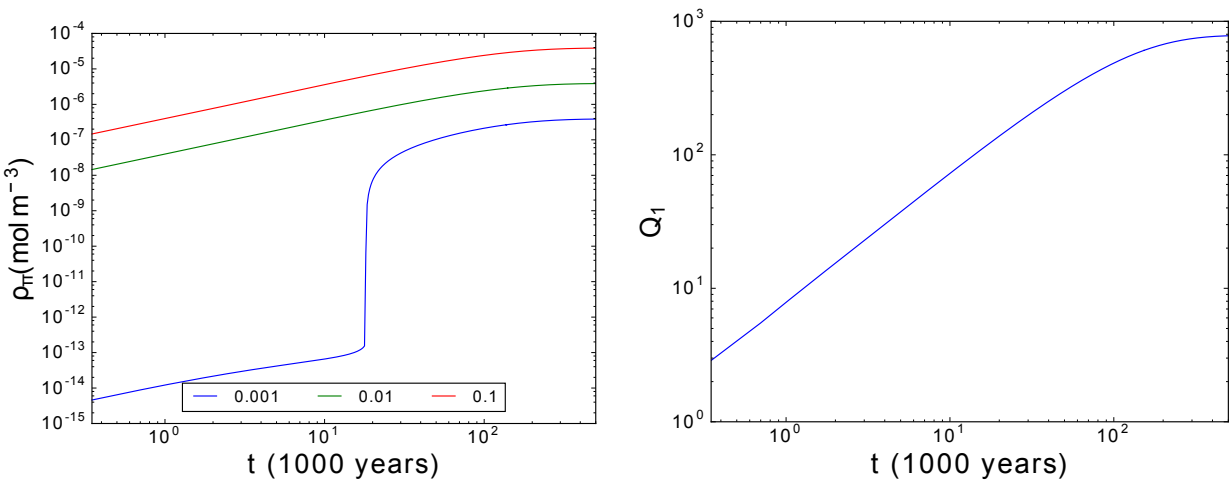


FIG. 5. a) Time evolution of the polymerase for initial concentration $\rho_r = \rho_p = 0.001, 0.01$ and 0.1 mol m^{-3} . b) Q_1 for initial concentration $\rho_r = \rho_p = 0.01 \text{ mol m}^{-3}$. Parameter values: $K_P^- = 4 \times 10^{-11} \text{ s}^{-1}$, $Z = 10^6 \text{ mol}^{-1} \text{ m}^3 \text{ s}^{-1}$, $\lambda = 0.15$.

all likelihood, were not identical to the ones existing when the polymerisation we are modelling occurred. In a second step, we departed from the set of values [Equation (21)] and found that in all cases investigated, varying these parameters

modified the critical concentrations of $\rho_{r,c}$ and $\rho_{p,c}$, but did not affect significantly the value of Q_1 while $Q_{2,10}$ remained extremely large.

More specifically, taking $K_P^- = 5.1 \times 10^{-6} \text{ s}^{-1}$ marginally increased the critical concentration

Table 2. Effect of initial peptide concentration on critical concentration

$\rho_p(\text{mol m}^{-3})$	$\rho_{r,c}(\text{mol m}^{-3})$
10^{-4}	2×10^{-3}
10^{-3}	10^{-3}
10^{-2}	8×10^{-4}
10^{-1}	7×10^{-4}

to $\rho_{r,c} = \rho_{p,c} = 0.0011 \text{ mol m}^{-3}$. Similarly, taking $k_{\text{step}} = 0.02 \text{ s}^{-1}$ increased slightly the critical concentrations: $\rho_{r,c} = \rho_{p,c} = 0.0017 \text{ mol m}^{-3}$. On the other hand, taking $Z = 10^8 \text{ mol}^{-1} \text{ m}^3 \text{ s}^{-1}$ lead to a decrease of the critical concentrations: $\rho_{r,c} = \rho_{p,c} = 0.0005 \text{ mol m}^{-3}$. Varying h_R to values as small as $1 \text{ mol}^{-1} \text{ m}^3 \text{ s}^{-1}$ did not change the critical concentrations.

In our model we have considered the concentrations of free amino acids ($\rho_p \equiv P_1$) and charged p-tRNA to be identical: $k_t \approx 1$ (see [Equation (7)]). To consider other values of k_t we only need to multiply the polymerisation rate of a peptide ($k_{P,1}^+$) by k_t as it is p-tRNAs that bind to XNA chains, not free amino acids. We have considered a large range of values for $k_{P,1}^+$ and found that for $k_{P,1}^+ = 10^{-5} \text{ mol}^{-1} \text{ m}^3 \text{ s}^{-1}$, the critical concentrations had not changed significantly while for $10^{-8} \text{ mol}^{-1} \text{ m}^3 \text{ s}^{-1}$, they increased to $\rho_{r,c} = \rho_{p,c} = 0.002 \text{ mol m}^{-3}$. This shows that taking much smaller values of k_t has a very small impact on our results and that having a concentration of charged p-tRNA much smaller than that of free amino acids would only increase marginally the

critical concentrations we have obtained using our original assumption.

The parameters on which the model is the most sensitive are K_R^+ and K_R^- . We found that for $K_R^+ = 4 \times 10^{-8} \text{ mol}^{-1} \text{ m}^3 \text{ s}^{-1}$, $\rho_{r,c} = \rho_{p,c} = 0.007 \text{ mol m}^{-3}$ and for $K_R^+ = 4 \times 10^{-9} \text{ mol}^{-1} \text{ m}^3 \text{ s}^{-1}$, $\rho_{r,c} = \rho_{p,c} = 0.05 \text{ mol m}^{-3}$. Similarly, for $K_R^- = 10^{-7} \text{ s}^{-1}$ we found that $\rho_{r,c} = \rho_{p,c} = 0.01 \text{ mol m}^{-3}$ and for $K_R^- = 10^{-6} \text{ s}^{-1}$ that $\rho_{r,c} = \rho_{p,c} \approx 0.05 \text{ mol m}^{-3}$. This shows that the spontaneous polymerisation of polynucleotide is essential to reach a minimum concentration of polynucleotides to kick start the whole catalysis process and that the stability of the polynucleotides plays an important role.

To investigate this, we have run simulations with $K_R^+ = 4 \times 10^{-8} \text{ mol}^{-1} \text{ m}^3 \text{ s}^{-1}$ for a fixed duration, τ_{pol} , after which K_R^+ was set to 0. We found that if τ_{pol} was long enough, the polymerisation of polypeptide and polynucleotide chains was identical to the one obtained when K_R^+ was not modified. When τ_{pol} was too short, on the other hand, one was only left with short polypeptide and polynucleotide chains in an equilibrium controlled by the spontaneous polymerisation and depolymerisation parameters. The minimum value for τ_{pol} depends on the concentrations ρ_r and ρ_p and the results are given in Table 3.

This shows that while K_R^+ is an important parameter in the process, what matters is to have a spontaneous generation of polynucleotides at the

Table 3. Effect of initial concentration of free nucleotides on time for production of polymerase

$\rho_r = \rho_p (\text{molm}^{-3})$	$\tau_{\text{pol}} (\text{years})$
0.001	18000
0.002	254
0.005	12.7
0.01	2.2

onset (Mechanism (A)). This then leads to the production of polypeptides, including polymerase (Mechanism (C)) and, once the concentration of polymerase is large enough, the catalysed production of polynucleotides (Mechanism (B)) dominates the spontaneous polymerisation.

We have also varied K_R^- once the system had settled and we found that for $\rho_r = \rho_p = 0.01 \text{molm}^{-3}$, K_R^- could be increased up to $6 \times 10^{-7} \text{s}^{-1}$ while still keeping a large amount of polymerase. Above that value, the polynucleotides are too unstable and one ends up again with mostly short polymer chains and $Q_1 \approx 1$.

We have also considered values of $L_{\text{max}} > 10$ and found that the main difference is a slight increase of the critical concentrations. For example, for $L_{\text{max}} = 11, 12$ and 15 , $\rho_{r,c} = \rho_{p,c}$ are respectively equal to $0.001, 0.0011$ and 0.0011molm^{-3} . At given concentrations Q_1 and ρ_π remain unchanged but $P_{L_{\text{max}}}^\pi$ decreases approximately by a factor of 40 each time L_{max} is increased by 1 unit.

We have also taken $L_{\pi\text{min}} = 4, 5$ and 6 and found that the critical concentrations were respectively $2 \times 10^{-5}, 2 \times 10^{-4}$ and $4 \times 10^{-4} \text{molm}^{-3}$, while ρ_π took the values of approximately $0.012, 2.6 \times$

10^{-3} and $3 \times 10^{-4} \text{molm}^{-3}$. Q_1 on the other hand remained constant.

A summary of the parameter values investigated outside the set [Equation (21)] and the corresponding critical concentrations are given in Table 4. Only one parameter was changed at a time. (see also the Supplementary Information section).

Table 4. Effect of various parameters on initial critical concentrations

MODIFIED PARAMETER	$\rho_{r,c} = \rho_{p,c} (\text{molm}^{-3})$
$K_P^- = 5.1 \times 10^{-6} \text{s}^{-1}$	1.1×10^{-3}
$k_{\text{step}} = 2 \times 10^{-2} \text{s}^{-1}$	1.7×10^{-3}
$Z = 10^8 \text{mol}^{-1} \text{m}^{-3} \text{s}^{-1}$	5×10^{-4}
$h_R = 1 \text{mol}^{-1} \text{m}^{-3} \text{s}^{-1}$	10^{-3}
$k_{P,1}^+ = 10^{-5} \text{mol}^{-1} \text{m}^3 \text{s}^{-1}$	10^{-3}
$k_{P,1}^+ = 10^{-8} \text{mol}^{-1} \text{m}^3 \text{s}^{-1}$	2×10^{-3}
$L_{\text{max}} = 15$	1.1×10^{-3}
$L_{\pi\text{min}} = 6$	4×10^{-4}
$L_{\pi\text{min}} = 5$	2×10^{-4}
$L_{\pi\text{min}} = 4$	2×10^{-5}
$K_R^+ = 4 \times 10^{-8} \text{mol}^{-1} \text{m}^3 \text{s}^{-1}$	7×10^{-3}
$K_R^+ = 4 \times 10^{-9} \text{mol}^{-1} \text{m}^3 \text{s}^{-1}$	5×10^{-2}
$K_R^- = 10^{-7} \text{s}^{-1}$	10^{-2}
$K_R^- = 10^{-6} \text{s}^{-1}$	0.19

Discussion

We describe a theoretical nucleopeptidic reciprocal replicator comprising a polynucleotide that templates the assembly of small p-tRNA

adapter molecules, most likely having mixed backbone architectures. These spontaneously arising p-tRNAs would have been bound to various classes of amino acids (possibly via weak stereochemical specificity), and a simple increase in local concentration mediated by binding to the p-Rib (in its most primitive version nothing much more than a mixed backbone architecture p-mRNA) could have driven polypeptide polymerisation. Once a template arose that coded for a peptide able to catalyze phosphodiester bond formation, this p-Rib could have templated assembly of its own complementary strand (and vice versa) and the self-replication cycle would have been complete. (See Figure 6 for a summary).

Starting from a single peptide and single polynucleotide, the IDA would quickly have become a distribution of related sequences of peptides and XNAs. We can imagine that over time, different p-Ribs encoding different peptides with additional functionalities could have appeared as the system evolved and that these p-Ribs may have subsequently fused together into larger molecules.

By imagining the IDA swiftly becoming a pool of molecules where variety within the “species” is maintained by the poor copying fidelity of a statistical operational code, should any mutation that stops replication arise, the other molecules in the pool would still function, ensuring continuity of the whole. Indeed this could have provided a

selective pressure for superior replicators. While our model does not directly consider less than perfect copying fidelity, it is not expected to have a major effect on our conclusions as copies with decreased performance would not be maintained as a significant proportion of the population and copies with increased performance would simply take over the role of main replicator.

The primordial operational code may only have required two bases per p-tRNA to deliver statistical proteins, while the catalytic requirements of the p-Pol are loose enough that a 7-residue peptide is a plausible lower length limit. This reduces the minimum length of the posited spontaneously arising p-Rib to just 14 nucleotides (assuming no spaces between codons). This is an optimistic length estimate, but given the available time and with molecular co-evolution, inorganic catalysts and geological PCR, considerably longer molecules may have been possible (Baaske et al 2007; Fishkis 2010). These p-Pols would act on p-Ribs and the crucial abiogenesis step would be the emergence of a 14-mer XNA that, in the context of the primordial operational code, happened to code for a peptide able to bind XNA and catalyze phosphodiester bond formation of base-paired nucleotides. Although the concentrations of various components are not known with certainty this does not seem an unreasonable proposition particularly given that functional peptides are

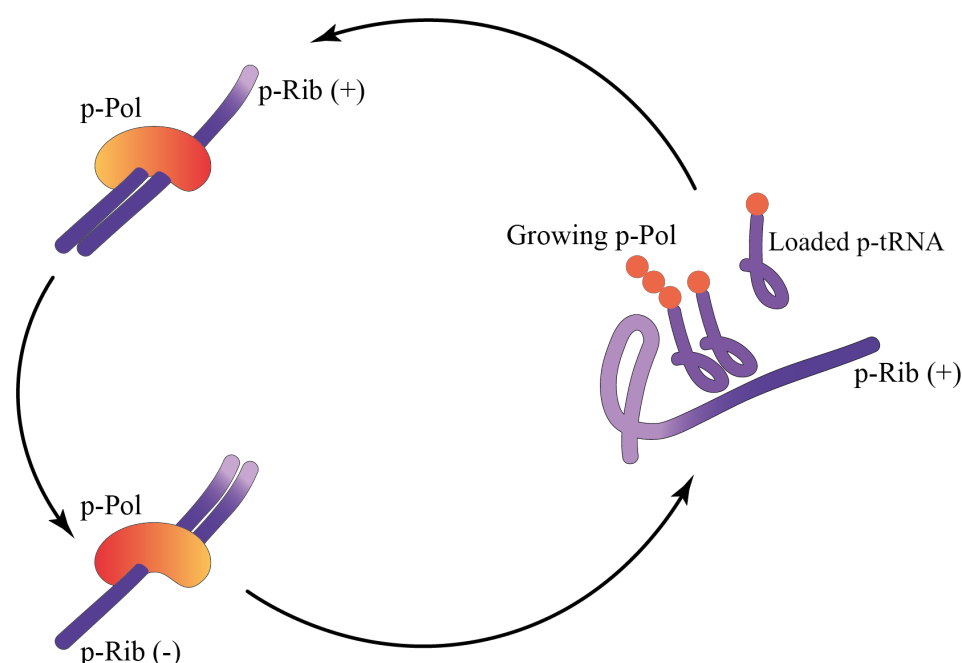


FIG. 6. The nucleopeptide Initial Darwinian Ancestor. In this cartoon model a short strand of XNA has the functionality of both a primordial p-mRNA and a p-Rib. Primitive XNA molecules loaded with amino acids (p-tRNA) bind to the p-Rib via codon-anticodon pairing. This allows adjacent amino acids to undergo peptide bond formation and a short peptide chain is produced. A certain peptide sequence is able to act as a primordial XNA-dependent XNA polymerase (p-Pol) able to copy both + and - p-Rib strands to eventually produce a copy of the p-Rib(+) strand.

known to occur in random sequences with surprising frequency (Keefe and Szostak 2001).

Our mathematical model showed that the most important parameters, apart from the concentration of loaded p-tRNA and polynucleotides, are the spontaneous polymerisation and depolymerisation of polynucleotides. It also shows that polynucleotides are first polymerised spontaneously and that these initial polynucleotides catalyse the production of the first polypeptides, including the polymerase. These polymerases can then generate further polynucleotides through catalysis. The stability gained by polymerases while being bound to polynucleotides ultimately leads to an increase of their relative concentration compared to the other polypeptides.

Overall, the hypothesis explains the coupling of polynucleotide and polypeptide polymerisation, the operational code and mutations in the p-Pol sequence that could eventually result in increased specificities leading to primitive DNA polymerases and RNA polymerases. No extraordinary exchanges of function are required and each molecule is functionally similar to its present-day analogue. Like all new abiogenesis theories, this IDA requires in vitro confirmation; in particular, the steps required for the primordial operational code to arise *ab initio* warrant close attention.

The idea that the ancestral replicator may have consisted of both nucleic acid and peptide components (the ‘nucleopeptide world’) is in itself not new, but compared to the RNA

world, has been somewhat neglected. We argue that molecular co-evolution of polynucleotides and peptides seems likely and cross-catalysis is known to be possible, for example in vitro selection experiments delivered RNA with peptidyl transferase activity after just nine rounds of a single selection experiments (Fishkis 2010; Zhang and Cech 1997). Inversely, Levy and Ellington produced a 17-residue peptide that ligates a 35 base RNA (Levy and Ellington 2003).

Nucleopeptide world research is relatively sparse, the data collected so far hint that cross-catalysis may be more efficient than autocatalysis by either peptides or nucleic acids. A self-replicating primordial system wherein RNA encoding for protein was replicated by a primordial RNA-dependent RNA polymerase which carried out the role of a replicative agent rather than as a transcriber of genes has previously been suggested (Leipe et al 1999), although in this case no further development of the concept to produce a self-contained replicating system was pursued. The merits of a “two polymerase” system where RNA catalyses peptide polymerisation and vice versa were succinctly explained by Kunin (Kunin 2000), although possible mechanisms and validity were not considered in detail. The possibility of a two polymerase system is also mentioned by van der Gulik and Speijer as part of a wider review of the co-evolution of peptides and RNA (van der Gulik

and Speijer 2015) but without a mathematical model.

Other origins of life hypotheses propose that the initial self-replicator did not consist of polynucleotides and/or peptides but was originally composed of different materials, most famously clay crystals (Cairns-Smith 1982). Such hypotheses are of interest but were not considered in this work as the IDA presented here does not require genetic takeover of one replication system by another and can be achieved using building blocks likely to have been present on the early earth and so appears more parsimonious. Our IDA hypothesis has tried to set out more rigorously the possible steps and processes whereby a nucleopeptide IDA could have arisen and could be tested experimentally.

Future experimental work that would support the nucleopeptide theory would be to provide evidence that the stereochemical hypothesis applies to the earliest occurring amino acids including those likely to have composed the active site of the p-Pol. Currently codon/anticodon binding to a number of amino acids has been shown (Yarus et al 2005) but is absent for the four earliest amino acids (Wolf and Koonin 2007). This may be due to their small sizes though even here possible solutions have been proposed (Tamura 2015).

It is important to note that we do not propose that the RNA world did not or could not exist, nor does this work necessarily suggest that a

self-replicating RNA polymerase did not exist (although our results suggest it to be unlikely), but rather that such a molecule did not directly lead to current living systems. Indeed the crucial role of RNA (more correctly, XNA) in our model is highlighted by the importance of K_R^+ , the rate of polymerisation of polynucleotide chains. We also do not dismiss any roles for ribozymes - for example it could well be that ribozymes were responsible for aminoacylation reactions (although this would inevitably raise the question of how such ribozymes were themselves replicated). Similarly (and with similar provisos), peptides alone could also have carried out supporting roles such as stabilising long XNA sequences or catalysing aminoacylation reactions. At its core however, we suggest that the ancestral replicator was nucleopeptidic with information storage function carried out by the XNA and polymerase function carried out by the peptide.

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References

- Angyan AF, Ortutay C, Gaspari Z. 2014. Are proposed early genetic codes capable of encoding viable proteins? *J. Mol. Evol.* 78:263-274.
- Baaske P, Weinert FM, Duhr S, Lemke KH, Russell MJ, Braun D. 2007. Extreme accumulation of nucleotides in simulated hydrothermal pore systems. *Proc. Natl. Acad. Sci. USA* 104:9346-9351.
- Biro JC, Benyo B, Sansom C, Szlavetz A, Fordos G, Micsik T, Benyo Z. 2003. A common periodic table of codons and amino acids. *Biochem. Biophys. Res. Commun.* 306:408-415.
- Bromley EHC, Channon K, Moutevelis E, Woolfson DN. 2008. Peptide and Protein Building Blocks for Synthetic Biology: From Programming Biomolecules to Self-Organized Biomolecular Systems. *ACS Chem. Biol.* 3:38-50.
- Cairns-Smith AG. 1982. Genetic takeover and the mineral origins of life. Cambridge University Press.
- Carny O, Gazit E. 2005. A model for the role of short self-assembled peptides in the very early stages of the origin of life. *FASEB J* 19:1051-1055.
- Cech TR. 2012. The RNA Worlds in Context. *Cold Spring Harb. Perspect. Biol.* 4:a006742.
- Da Silva L, Maurel MC, Deamer D. 2015. Salt-promoted synthesis of RNA-like molecules in simulated hydrothermal conditions. *J. Mol. Evol.* 80:86-97.
- Danger G, Plasson R, Pascal R. 2012. Pathways for the formation and evolution of peptides in prebiotic environments. *Chem. Soc. Rev.* 41:5416-5429.
- Di Giulio M. 1997. On the RNA world: Evidence in favor of an early ribonucleopeptide world. *J. Mol. Evol.* 45:571-578.
- Ekland E.H. and Bartel, D.P. 1996. RNA-catalysed RNA polymerization using nucleoside triphosphates. *Nature.* 382:373-376.
- Dixit SB, Arora N, Jayaram B. 2000. How Do Hydrogen Bonds Contribute to Protein-DNA Recognition?. *J.*

- Biomol. Struct. Dyn.* 17:109-112.
- Fishkis M. 2010. Emergence of Self-Reproduction in Cooperative Chemical Evolution of Prebiological Molecules. *Origins Life Evol. B.* 41:261-275.
- Fletcher JM, Harniman RL, Barnes FR, Boyle AL, Collins A, Mantell J, Sharp TH, Antognozzi M, Booth PJ, Linden N, et al. 2013. Self-assembling cages from coiled-coil peptide modules. *Science* 340:595-599.
- Fox SW. and Harada K. 1958. Thermal copolymerization of amino acids to a product resembling protein. *Science* 128:1214.
- Giel-Pietraszuk M, Barciszewski J. 2006. Charging of tRNA with non-natural amino acids at high pressure. *FEBS J.* 273:3014-3023.
- Giri V, Jain S. 2012. The Origin of Large Molecules in Primordial Autocatalytic Reaction Networks. *Plos One* 7:e29546.
- Gounaris Y. 2011. An evolutionary theory based on a protein-mRNA co-synthesis hypothesis. *J. Biol. Res. Thessalon.* 15:3-16.
- Herschy B, Whicher A, Camprubi E, Watson C, Dartnell L, Ward J, Evans JRG, Lane N. 2014. An origin-of-life reactor to simulate alkaline hydrothermal vents. *J. Mol. Evol.* 79:213-227.
- Ikehara K. 2002. Origins of gene, genetic code, protein and life: comprehensive view of life systems from a GNC-SNS primitive genetic code hypothesis. *J. Biosci.* 27:165-186.
- Ikehara K. 2005. Possible steps to the emergence of life: The [GADV]-protein world hypothesis. *Chem. Rec.* 5:107-118.
- Illangasekare M, Sanchez G, Nickles T, Yarus M. 1995. Aminoacyl-RNA synthesis catalyzed by an RNA. *Science* 267:643-647.
- Issac R, Chmielewski J. 2002. Approaching exponential growth with a self-replicating peptide. *J. Am. Chem. Soc.* 124:6808-6809.
- Iyer L, Koonin E, Aravind L. 2003. Evolutionary connection between the catalytic subunits of DNA-dependent RNA polymerases and eukaryotic RNA-dependent RNA polymerases and the origin of RNA polymerases. *BMC Struct. Biol.* 3:1.
- Keefe AD, Szostak JW. 2001. Functional proteins from a random-sequence library. *Nature* 410:715-718.
- Kemeny J. 1955. Man Viewed as a Machine. *Sci. Am.* 192:58-68.
- Knight RD, Landweber LF. 2000. Guilt by association: The arginine case revisited. *RNA.* 6:499-510.
- Kochavi E, Bar-Nun A, Fleminger G. 1997. Substrate-directed formation of small biocatalysts under prebiotic conditions. *J. Mol. Evol.* 45:342-351.
- Koonin EV, Novozhilov AS. 2009. Origin and Evolution of the Genetic Code: The Universal Enigma. *Iubmb Life.* 61:99-111.
- Koonin EV. (1991). The phylogeny of RNA-dependent RNA polymerases of positive-strand RNA viruses. *J. Gen. Virol.* 72:2197-2206.
- Kunin V. 2000. A system of two polymerases—a model for the origin of life. *Origins Life Evol. B.* 30:459-466.
- Kurland CG. 2010. The RNA dreamtime. *Bioessays* 32:866-871.
- Lahav N. 1995. The RNA-World and Coevolution Hypotheses and the Origin of Life - Implications, Research Strategies and Perspectives. *Origins Life Evol. B.* 23:329-344.
- Lee DH, Granja JR, Martinez JA, Severin K, Ghadiri MR. 1996. A self-replicating peptide. *Nature* 382:525-528.
- Lehmann J, Reichel A, Buguin A, Libchaber A. 2007. Efficiency of a self-aminoacylating ribozyme: effect of the length and base-composition of its 3' extension. *RNA* 13:1191-1197.
- Leipe DD, Aravind L, Koonin EV. 1999. Did DNA replication evolve twice independently? *Nucleic Acids Res.* 27:3389-3401.
- Leman L, Orgel L, Ghadiri MR. 2004. Carbonyl sulfide-mediated prebiotic formation of peptides. *Science* 306:283-286.

- Levy M, Ellington AD. 2003. Peptide-templated nucleic acid ligation. *J. Mol. Evol.* 56:607-615.
- Liu Z, Beaufils D, Rossi JC, Pascal R. 2014. Evolutionary importance of the intramolecular pathways of hydrolysis of phosphate ester mixed anhydrides with amino acids and peptides. *Sci. Rep.* 4:7440.
- Manning MC, Illangasekare M, Woody RW, 1988. Circular dichroism studies of distorted alpha-helices, twisted beta-sheets, and beta turns. *Biophys. Chem.* 31:77-86.
- Martin W, Baross J, Kelley D, Russell MJ. 2008. Hydrothermal vents and the origin of life. *Nat. Rev. Microbiol.* 6:805-814.
- Maury CPJ. 2009. Self-Propagating β -Sheet Polypeptide Structures as Prebiotic Informational Molecular Entities: The Amyloid World. *Origins Life Evol. B.* 39:141-150.
- McCleskey SC, Griffin MJ, Schneider SE, McDevitt JT, Anslyn EV. 2003. Differential Receptors Create Patterns Diagnostic for ATP and GTP. *J. Am. Chem. Soc.* 125:1114-1115.
- Miller, SL. 1997. Peptide nucleic acids and prebiotic chemistry. *Nat Struct Biol* 4, 167-169.
- Milner-White EJ, Russell MJ. 2011. Functional capabilities of the earliest peptides and the emergence of life. *Genes* 2:671-688.
- Moore PB, Steitz TA. 2003. After the ribosome structures: How does peptidyl transferase work? *RNA* 9:155-159.
- Morgens DW. 2013. The protein invasion: a broad review on the origin of the translational system. *J. Mol. Evol.* 77:185-196.
- Nelson KE., Levy M. and Miller SL. 2000. Peptide nucleic acids rather than RNA may have been the first genetic molecule. *Proceedings of the National Academy of Sciences* 97, 3868-3871.
- Noller HF. 2012. Evolution of Protein Synthesis from an RNA World. Cold Spring Harb. *Perspect. Biol.* 4:a003681.
- Orgel LE, 1989. The origin of polynucleotide-directed protein synthesis. *J. Mol. Evol.* 29:465-474.
- Patel BH, Percivalle C, Ritson DJ, Duffy CD, Sutherland JD. 2015. Common origins of RNA, protein and lipid precursors in a cyanosulfidic protometabolism. *Nat. Chem.* 7:301-307.
- Paul N, Joyce GF. 2004. Minimal self-replicating systems. *Curr. Opin. Chem. Biol.* 8:634-639.
- Pinheiro VB, Taylor AI, Cozens C, Abramov M, Renders M, Zhang S, Chaput JC, Wengel J, Peak-Chew SY, McLaughlin SH, et al. 2012. Synthetic genetic polymers capable of heredity and evolution. *Science* 336:341-344.
- Regan L, DeGrado WF, 1988. Characterization of a helical protein designed from first principles. *Science* 241:976-978.
- Riddle DS et al., 1997. Functional rapidly folding proteins from simplified amino acid sequences. *Nat. Struct. Biol.* 4:805-809.
- Robertson MP, Joyce GF. 2012. The Origins of the RNA World. Cold Spring Harb. *Perspect. Biol.* 4:a003608.
- Rodin AS, Szathmary E, Rodin SN. 2011. On origin of genetic code and tRNA before translation. *Biol. Direct* 6:14.
- Root-Bernstein R, and Root-Bernstein M. 2016. The ribosome as a missing link in prebiotic evolution II: ribosomes encode ribosomal proteins that bind to common regions of their own mRNAs and rRNAs. *J. Theor. Biol.* 397:115-127.
- Roviello G. et al. 2009. Solid phase synthesis and RNA-binding studies of a serum-resistant nucleo-epsilon-peptide. *J Pept Sci* 15, 155-160.
- Ruiz-Mirazo K, Briones C, de la Escosura A. 2014. Prebiotic systems chemistry: new perspectives for the origins of life. *Chem. Rev.* 114:285-366.
- Saladino R, Botta G, Pino S, Costanzo G, Di Mauro E. 2012. Genetics first or metabolism first? The formamide clue. *Chem. Soc. Rev.* 41:5526-5565.
- Schimmel P, Henderson B. 1994. Possible role of aminoacyl-RNA complexes in noncoded peptide synthesis and origin of coded synthesis. *Proc. Natl. Acad. Sci. USA* 91:11283-11286.

- Schneider SE, O'Nei SN, Anslyn EV. 2000. Coupling Rational Design with Libraries Leads to the Production of an ATP Selective Chemosensor. *J. Am. Chem. Soc.* 122:542-543.
- Sievers A, Beringer M, Rodnina MV, Wolfenden R. 2004. The ribosome as an entropy trap. *Proc. Natl. Acad. Sci. USA* 101:7897-7901.
- Tamura K. 2015. Beyond the Frozen Accident: Glycine Assignment in the Genetic Code. *J. Mol. Evol.* 81:69-71.
- Tamura K, Schimmel P. 2003. Peptide synthesis with a template-like RNA guide and aminoacyl phosphate adaptors. *Proc. Natl. Acad. Sci. USA* 100:8666-8669.
- Trevino SG, Zhang N, Elenko MP, Luptak A, Szostak JW. 2011. Evolution of functional nucleic acids in the presence of nonheritable backbone heterogeneity. *Proc. Natl. Acad. Sci. USA* 108:13492-13497.
- Turk RM, Chumachenko NV, Yarus M. 2010. Multiple translational products from a five-nucleotide ribozyme. *Proc. Natl. Acad. Sci. USA* 107:4585-4589.
- van der Gulik P. and Speijer D. 2015. How Amino Acids and Peptides Shaped the RNA World. *Life* 5, 230.
- Vetsigian K, Woese C, Goldenfeld N. 2006. Collective evolution and the genetic code. *Proc. Natl. Acad. Sci. USA* 103:10696-10701.
- Vidonne A, Philp D. 2009. Making Molecules Make Themselves - the Chemistry of Artificial Replicators. *Eur. J. Org. Chem.* 2009:593-610.
- Woese CR. 1965. On the evolution of the genetic code. *Proc. Natl. Acad. Sci. USA* 54:1546-1552.
- Wolf YI, Koonin EV. 2007. On the origin of the translation system and the genetic code in the RNA world by means of natural selection, exaptation, and subfunctionalization. *Biol. Direct* 2:14.
- Yarus M, Caporaso JG, Knight R. 2005. Origins of the genetic code: The escaped triplet theory. *Annu. Rev. Biochem.* 74:179-198.
- Yarus M, Widmann JJ, Knight R. 2009. RNAamino acid binding: a stereochemical era for the genetic code. *J. Mol. Evol.* 69:406-429.
- Yarus M. 2011. Getting Past the RNA World: The Initial Darwinian Ancestor. *Cold Spring Harbor Perspect. Biol.* 3:a003590.
- Zhang BL, Cech TR. 1997. Peptide bond formation by in vitro selected ribozymes. *Nature* 390:96-100.